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THYMECTOMY AND RENAL HOMOTRANSPLANTATION

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SUMMARY

The course of forty-six consecutive patients were studied for 3½–5 years after renal homotransplantation from related and unrelated donors. Transthoracic thymectomy was performed before transplantation in twenty-four cases; the other twenty-two recipients served as controls. A similar spectrum of donor-recipient lymphocyte antigen compatibility was present in both the test and control series.

In both the related and non-related cases, there was no clinical evidence that the patients with thymectomy had either an early or late advantage in terms of survival, reduced drug dosages, or quality of renal function. However, pathologic studies with light and electron microscopy and with immunofluorescence revealed that the homografts in the thymectomized patients had fewer and less severe lesions of the kind that would be expected to limit the functional life time of these organs.

INTRODUCTION

In both animals and man there is incontrovertible evidence that the thymus gland has an important influence upon the development of immunologic competence. Rodents deprived of this organ during the embryonic or early newborn period quickly develop lymphoid hypoplasia, lose their capacity to react against a variety of antigens, suffer from a wasting syndrome, and have increased susceptibility to infection (Miller, 1961, 1962b; Martinez *et al.*, 1962). In addition, they have a greatly reduced ability to reject skin homografts. A comparable syndrome of immunologic crippling has been described in children born with thymic aplasia (Good *et al.*, 1966). In both the foregoing circumstances, thymic function was either never present or was eliminated in the early neonatal period. In contrast, thymectomy upon normal adult animals (Fichtelius, Laurell & Philipsson, 1961) or man (Zollinger *et al.*, 1964) does not have these striking immediate effects.

In 1961, Miller (1962a) demonstrated that an abrupt loss of immunologic responsiveness could follow adult thymectomy under special conditions of testing. Adult mice were pro-

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vided with skin homografts after sublethal total body irradiation. Animals with prior thymectomy retained their transplants for much longer periods than their non-thymectomized mates. It appeared that recovery from a profound immunologic depression recapitulated some of the events of normal intrauterine and neonatal life in being at least partially thymus-dependent.

Shortly after Miller's report, eight adult humans were subjected to thymectomy at the University of Colorado, 14–85 days before renal homotransplantation. Four of the recipients died within a few months. The other four had function of their new kidneys for at least 5½ years. In one case, the homograft eventually failed and was replaced; the other three transplanted organs have continued to function satisfactorily for 6½–6¾ years. All four of these patients remain healthy and have exhibited neither an unusual disposition to infection nor other manifestations of a severe immunologic deficiency state.

The unusually untroubled early and late courses of these four patients have been noted in past reports (Starzl *et al.*, 1963; Starzl, 1964; Starzl *et al.*, 1965), and attention was drawn to the possible contributing role of the thymectomies. However, it was emphasized that the results could be explained solely by favourable histocompatibility matches fortuitously obtained between these recipients and their donors (Starzl *et al.*, 1965). In order to clarify this issue, a formal study of the effect of thymectomy was carried out in forty-six more patients who were treated with renal homotransplantation between October, 1964 and June, 1966.

METHODS

Clinical studies

All kidneys were provided by living volunteers (Table 1). In each case, lymphocyte antigen typing was carried out by Terasaki (Terasaki, Vredevoe & Mickey, 1967), using a large panel of antisera. Shortly after the series had been completed, the matches were graded at one sitting from A to D, giving special weight to the 6 HLA antigen groups that became internationally accepted in 1967. Since then, there have been substantial improvements in the accuracy and completeness of typing. However, the original classification of results was retained because not all patients and their donors could be re-examined. An A match indicated identity of the 6 HLA antigens with incompatibilities in less than 5% in the total panel of test antisera. B, C, and D designations indicated progressively less satisfactory combinations. With all C or D matches, there were incompatibilities of one or more of the major HLA groups.

A decision for or against thymectomy was made by random selection from appropriately marked cards. The thymectomies were performed 8–112 days (average 22 days) before transplantation, through an incision in the second or third left intercostal space. Care was taken to excise the cervical thymic tissue. On the day of transplantation, the recipient spleens and diseased kidneys were removed.

Immunosuppressive therapy was with azathioprine, prednisone, and occasionally with intravenous actinomycin C and local homograft irradiation (Starzl, 1964). Prednisone was usually started on the day of operation in a dose of 0·5–1·0 mg/kg per day. This was increased when necessary to control rejection. Four recipients of related homografts, two with thymectomy and two without, were also given horse antihuman-lymphocyte globulin (ALG) injections intramuscularly for the first 4 postoperative months (Starzl *et al.*, 1967) in addition to azathioprine and prednisone.

The results of thymectomy were evaluated on the basis of mortality, the quality of renal function, the quantities of immunosuppressive drugs necessary to retain this function, the numbers of peripheral white blood cells and lymphocytes, and the pathologic changes that occurred in the renal homografts. The data on drug dosage, renal function, and white cell counts were compiled on a weekly basis and converted for purposes of statistical analysis to daily averages during the first 10 postoperative months. In so doing, patients who failed to live this long were eliminated. This excluded cases in which early death was due to technical complications, sepsis, uncontrolled rejection, or a combination of these things, and left a pool of the kind of cases in which a delayed benefit from thymectomy might be expected.

TABLE 1. Antigen matches performed by Terasaki between donors and recipients in the thymectomy and non-thymectomy groups
(See text for Definition of A–D rating)

		Related donor					Non-related donor					
	Match‡	A	B	C	D	Total	A	B	C	D	Total	Grand total
Thymectomy		4	5	5	0	14*	0	3	6	1	10	24
Non-thymectomy		2	8	4	1	15†	1	1	5	0	7	22
Total		6	13	9	1	29	1	4	11	1	17	46

* 5 parents, 8 siblings, 1 uncle.

† 10 parents, 5 siblings.

‡ For reasons discussed in the text, these letter grades probably indicate somewhat better matches than were actually present, since the examination in 1964 to 1966 had to be carried out with less complete knowledge of the HLA antigens than is available today. However, the original grades have been retained since the conditions of testing upon which they were based were relatively consistent throughout the interval when the transplantations were performed.

Pathologic studies

Tissue processing. Tissue was obtained from all forty-six grafted kidneys either at necropsy or as a result of open biopsy. A portion of each specimen was fixed in 10% neutral formalin, embedded in paraffin wax, serially sectioned, and stained with haematoxylin and eosin, periodic acid-Schiff reagent, Weigert's stain for elastic tissue, and methyl green-pyronin. Another part was immediately cut into small cubes, fixed in Palade's buffered osmium tetroxide, and embedded in Epon 812. Sections, 0·5 μ thick, cut from these blocks were stained with azure II and examined by light microscopy. Very thin sections for electron microscopy were stained with lead hydroxide and examined in a Phillips 300 electron-microscope. A third piece of tissue was snap-frozen in isopentane in liquid nitrogen and sectioned in a cryostat at 4 μ, and the unfixed tissue was stained by the direct immunofluorescent method with fluorescein-labelled antisera (Seegal *et al.*, 1965).

Preparation of antisera for immunofluorescence. Antisera, labelled with fluorescein isothiocyanate, were prepared against the following: human IgG and IgM, both of which were isolated by diethylaminoethyl (DEAE) Sephadex A-50 chromatography (Fahey, 1962); human B1C/B1A-globulins (parts of C'3), which were prepared by a column chromato-

graphic method (Müller-Eberhard *et al.*, 1960); human C1q (11 S protein) purified according to the procedure of Morse & Christian (1964); human fibrinogen isolated by the method of Laki (1951); human albumin; rabbit γ-globulin; horse γ-globulin; and a heat-killed suspension of group A type 12 streptococci. The antisera to human IgG, IgM, and B1C/B1A-globulins were prepared in goats and rabbits; the antiserum to rabbit γ-globulin was

TABLE 2. Graded morphological abnormality in renal homografts in thymectomized patients*

Case number	Tissue match*	Type of histological abnormality†					Type of glomerular capillary deposit				Times of tissues (to nearest month)
		1	2	3	4	5	IgG	IgM	C′	Fib	
					Related						
65	A	0	0	0	0	0	0	0	0	0	25
66	A	0	1	0	1	1					2
68	B	2	1	1	1	0	0	2	0	0	23
76	C	0	3	2	3	3					10
84	C	2	0	1	1	1	0	0	0	0	30
89	A	4	2	1	2	1	2	3	3	1	28, 30
93	B	1	2	1	1	0	0	2	0	0	26
97	C	1	0	0	1	1					6
100	C	1	0	3	1	1	0	0	0	0	21
103	B	0	0	1	0	0	0	0	0	0	19
104	C	0	0	0	0	0					1
105	B	0	1	0	1	0					10
107	B	1	1	0	2	1	0	2	0	1	4, 31
109	A	1	0	0	0	0	0	0	0	0	4, 30
					Non-related						
67	B	2	2	1	2	0	0	0	0	0	23, 45
70	B	3	2	1	2	1	0	3	3	0	22
72	C	0	0	0	0	0					2
77	C	0	2	0	1	1					17
82	D	0	0	1	1	1					3
86	C	2	0	0	1	0	0	0	0	0	28
87	C	0	0	0	0	0	0	0	0	0	28
88	C	0	0	1	0	1					5
91	C	0	1	2	2	1	0	0	0	0	26
92	B	0	1	0	0	1					2
Cumulative score		20	19	16	23	15	2	12	6	2	
Mean		0·83	0·79	0·67	0·96	0·63	0·14	0·89	0·43	0·14	

* See text for details of both histologic and histocompatibility grading systems.

† Type 1 = Glomerular capillary basement membrane thickening; Type 2 = Thickening of intima of interlobular arteries; Type 3 = Interstitial fibrosis—superficial subcapsular; Type 4 = Interstitial fibrosis—generalized; Type 5 = Mononuclear cell infiltration.

prepared in a duck; and those to human C1q, human albumin, equine globulin, and type 12 streptococci were prepared in rabbits. Most of these antisera yielded single precipitin lines against their antigens in whole human serum by immunoelectrophoresis and by the agar double gel diffusion method (May & Rawlins, 1962). The fluorescein labelling was performed

as described by Riggs *et al.* (1958). The labelled antisera were passed through a Sephadex G-25 column before storing and immediately before use were absorbed with mouse liver powder. The antisera to human albumin and to rabbit γ-globulin were used as control stains, and in no instance was positive glomerular binding obtained. The specificity of the fluorescence was established by using an inhibition test in which the sections were pretreated with unlabelled antisera. Sections were also treated with conjugated antisera that had been absorbed with their specific antigens. Unfixed sections from three apparently normal unused donor kidneys from very recently dead subjects were treated with the fluorescein-labelled antisera. There was no glomerular localization of immunoglobulins, complement, or fibrinogen.

Assessment of histopathological and immunopathological changes. The presence or absence of the following five major histopathological and ultrastructural features was determined in all the grafted kidneys: (1) thickening of the glomerular capillary basement membranes, (2) thickening of the intima of the interlobular arteries, (3) superficial subcapsular interstitial fibrosis, (4) generalized interstitial fibrosis, (5) infiltration of the interstitium of the graft by mononuclear cells. In twenty-eight of the grafted kidneys the presence or absence of deposits of immunoglobulins G and M, complement (C1q and B1C/B1A-globulin) and fibrinogen was also noted.

The lesions and deposits were arbitrarily graded in severity from 1 to 4. Illustrations of each of the histopathological and immunopathological alterations have already been published (Ogden *et al.*, 1967; Porter *et al.*, 1968).

The histopathological and immunopathological gradings were determined by two of us (K.A.P. and G.A.A.) geographically separated from the patients and the other investigators and without prior knowledge of the results of the functional tests and without knowing whether an individual specimen was from a thymectomized or non-thymectomized patient.

Statistical techniques used to evaluate results. The graded lesions present in the grafted kidneys from the thymectomized were compared with those in the non-thymectomized patients. The significance of the difference between the mean values for the various abnormalities was tested with Student's *t*-test.

RESULTS

Antigen matches

Considering A or B to be good matches and C or D to be poor ones, the histocompatibility spectrum proved to be comparable in the thymectomy as opposed to the non-thymectomy cases (Tables 1–3).

Complications of thymectomy

Thymectomies were performed in twenty-four of the forty-six cases. There were two serious complications. The chest incision of one patient dehisced 9 days postoperatively and healed uneventfully after secondary closure. In another case, an apical hematoma in the left hemithorax was excised transthoracically 56 days after the thymectomy. Recovery from the decortication was uncomplicated.

Survival after intrafamilial transplantation

After a follow-up of 3½–5 years, seventeen of the twenty-nine consanguineous homografts

(59%) are still functioning adequately. The eventual mortality in the series of fourteen thymectomized recipients was 50%, compared to 33% in the non-thymectomized patients (Fig. 1). By analysis of survivorship, the difference was not statistically significant.

TABLE 3. Graded morphological abnormality in renal homografts in non-thymectomized patients*

Case number	Tissue match*	Type of histological abnormality†					Type of glomerular capillary deposit				Times of tissues (to nearest month)
		1	2	3	4	5	IgG	IgM	C′	Fib	
					Related						
73	B	4	3	2	4	1	3	4	3	4	21, 26
75	C	2	3	3	3	2	2	1	1	2	21
79	C	0	4	4	3	1					9
81	C	0	0	0	1	0					1
83	B	0	0	0	1	1					8
85	B	3	2	0	3	1	1	2	2	0	30
90	B	1	1	0	1	1	0	2	0	0	27
94	B	2	2	2	2	1	0	2	0	0	26
95	D	0	2	2	1	1					12
96	C	1	0	0	1	0	0	0	0	0	25
101	A	0	2	2	2	2	0	0	0	0	20
102	B	4	3	2	3	2	0	0	0	0	20
106	B	2	0	1	1	3	1	3	0	1	18
108	A	3	1	1	1	1	3	0	0	0	4, 29
110	B	4	2	2	2	2	3	2	3	0	4, 28
					Non-related						
69	B	0	0	0	0	1					0
71	C	3	4	3	4	1	0	2	2	0	22, 32
74	A	0	0	0	1	1					2
78	C	2	0	0	0	1	0	0	0	1	31, 50
80	C	0	2	1	1	4					10
98	C	0	0	0	0	0					1
99	C	0	0	1	0	1	0	2	0	0	23
Cumulative score		31	31	26	35	28	13	20	11	8	
Mean		1·41	1·41	1·18	1·59	1·27	0·93	1·43	0·79	0·57	

* See text for details of both histologic and histocompatibility grading systems.

† Type 1 = Glomerular capillary basement membrane thickening; Type 2 = Thickening of intima of interlobular arteries; Type 3 = Interstitial fibrosis—superficial subcapsular; Type 4 = Interstitial fibrosis—generalized; Type 5 = Mononuclear cell infiltration.

Three patients, two with and one without thymectomy, were lost after the first post-operative year. The deaths in the thymectomy group were caused by reticulum cell sarcoma (30 months) (Penn *et al.*, 1969) and by chronic Torula meningitis and renal failure (33 months); interpretation of the reason for deterioration of homograft function in the latter case was obscured by the need for long term therapy with amphoteracin B. The non-thymectomized recipient died after the kidney had undergone chronic rejection and had been removed after 24 months.

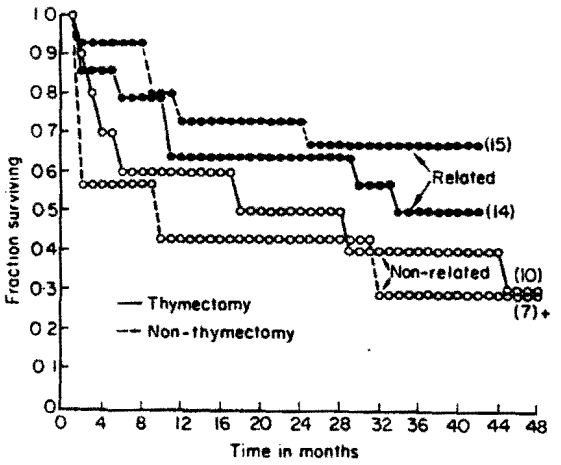

FIG. 1. Survival curves after renal homotransplantation in patients who received kidneys from related (solid circles) or non-related (open circles) donors. The followup period after the consanguineous transplantations has been 3½–5 years. With the non-related cases, the minimum followup has been 4 years. The cross indicates a death after the time covered by the graph. Thymectomy had no statistically significant effect upon survival.

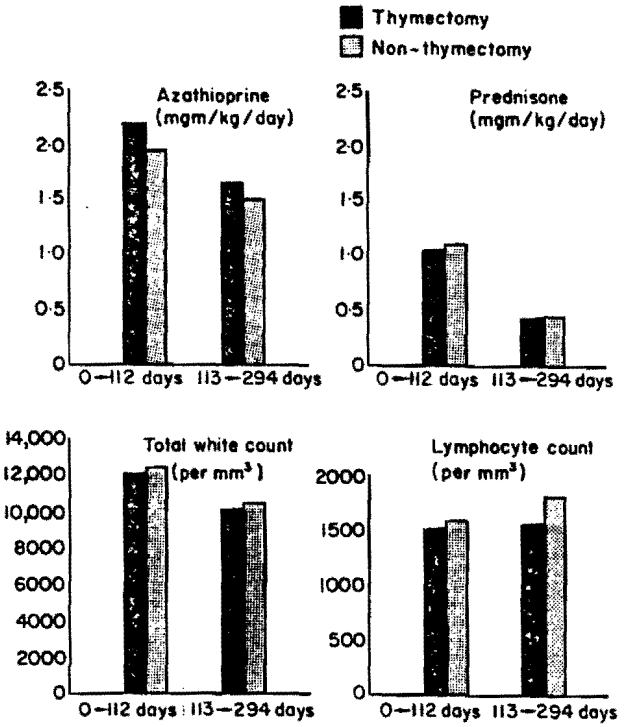

FIG. 2. Drug doses and white blood cell counts during the first 16 weeks and the subsequent 6 months in twenty-three recipients of related renal homografts. Patients who did not live through the period of study were excluded from the analysis. Eleven of the included recipients had received thymectomy and the other twelve had not. The differences in the control and thymectomy groups were not statistically significant.

Survival after transplantation from non-relatives

By the end of the fourth postoperative year, there were only five (30%) of the seventeen recipients of non-related homografts still living with function of their originally transplanted kidneys; an additional patient died after 52 months. During the first 4 years, the survival rate of the ten patients who had thymic excision remained approximately the same as that of the other seven who did not have this adjuvant procedure (Fig. 1).

In the combined series of seventeen cases, there were nine recipients (53%) who had life sustaining homograft function at the end of 1 year. Five of these patients, three with and two without thymectomy, died subsequently. The late deaths in the thymectomy group were caused by hepatitis (17½ months), a biopsy wound infection (28½ months), and quadriplegia (44½ months); renal function continued until just before death but at a reduced level. The two non-thymectomized recipients died at 31½ and 52 months. The homograft in the first case had become anuric. The second patient died in a distant city of acute pulmonary edema which was apparently secondary to renal failure despite the fact that his last measured creatinine clearance was 40 ml/min.

Immunosuppression

There was not a significant difference in the quantities of immunosuppressive agents given to patients of the test or control series. This is illustrated in Fig. 2 for the eleven thymectomized and the twelve non-thymectomized recipients of consanguineous homografts who lived for at least 294 days. The former patients received slightly more azathioprine but the prednisone doses were slightly higher in the latter group. These modest differences were present both during the first 16 postoperative weeks and for the ensuing 6 months. Similarly, there was no apparent effect of thymectomy in the non-related series.

White blood cell counts

In the related cases, the total white cell and peripheral lymphocyte counts determined in a research haematology laboratory were similar with or without thymectomy, both in the early and later postoperative periods (Fig. 2). The slightly lower counts in the patients with thymectomy were not statistically significant. In the smaller numbers of non-related cases, an effect of thymectomy was also not apparent.

Renal function

The average blood urea nitrogens, creatinines, and creatinine clearances in the aforementioned related transplantations were not influenced by the prior performance of thymectomy either during the first 16 postoperative weeks or in the subsequent 6 months (Fig. 3). The same was true for the non-related cases.

Long after transplantation, there was not a clear difference in the stability of renal function in the thymectomy versus the non-thymectomy series. In the ultimately unsuccessful cases, examples were cited earlier of secondary kidney deterioration in both groups, although this led to frank renal failure more frequently in recipients still possessing thymus glands. The twenty-one patients who remain alive after 3½–5 years have not had loss of homograft function since the twelth postoperative month; ten had thymectomy and eleven did not.

Patients treated with ALG

The last four recipients in the series were treated with adjuvant ALG for the first 4

postoperative months (Starzl *et al.*, 1967). Thymectomy was carried out in two of these cases and omitted in the other two. The donors were siblings and the histocompatibility matches were graded A or B. After 3½ years the four patients have excellent and approximately equal homograft function.

Pathologic studies

In Tables 2 and 3 are illustrated the graded histopathologic features in the renal transplants of the thymectomized and non-thymectomized patients and the presence or absence of immunoglobulins, complement, and fibrinogen in the glomeruli of these grafts. In both the related and unrelated patients the mean graded cumulative score for IgG deposits on the glomerular capillary basement membranes of the grafts was significantly less in the thymectomized patients ($P<0{\cdot}05$). The mean score for cellular infiltration of the interstitium

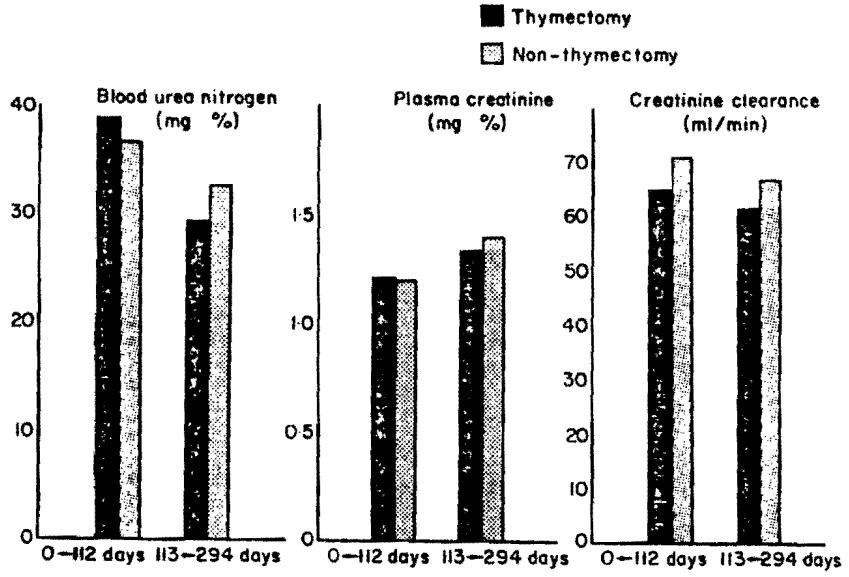

FIG. 3. Measures of renal function in the same patients and for the same time intervals as in Fig. 2. Note the lack of differences between the control and thymectomy groups.

was also less after thymectomy ($P<0{\cdot}02$). These differences seemed real since in both the thymectomy and non-thymectomy groups the numbers of autopsied and biopsied cases, the times of sampling, and the average grade of tissue typing were much the same. The differences were greatest in the tissues studied 2 or more years after renal transplantation. The lower scores for the seven other features in the grafts from the thymectomized patients either did not attain statistical significance or were of marginal significance.

DISCUSSION

There is strong evidence that excision of the thymus in adult mice, rats, and hamsters causes a slowly developing loss in immunologic reactivity. This has been shown in otherwise unaltered animals (Jeejeebhoy, 1965; Metcalf, 1965; Miller, 1965; Taylor, 1965; Sherman,

1966). The process can be magnified in skin homotransplantation experiments in rodents if immunosuppressive therapy is also given either with total body irradiation (Miller, 1962a) or with antilymphocyte serum (Jeejeebhoy, 1965; Monaco, Wood & Russell, 1965).

Efforts to reproduce these findings in dogs have been unsuccessful. Thymectomy, even when performed in the puppy neonatal period, has not been proved to cause an easily demonstrable loss of immunologic reactivity (Kelley, 1963; Fisher *et al.*, 1965; Levin, Beattie & Economou, 1966), even though there may be lymphoid involution (Tilney, Beattie & Economou, 1965). Moreover, the combination of thymectomy and heterologous antilymphocyte serum (ALS) or globulin (ALG) has been evaluated after canine renal homotransplantation in our laboratories (Starzl *et al.*, 1967) and by Atai & Kelley (1966). No additive effect of thymic excision could be seen, a conclusion also reached by Hume & Wolf (1967) in their studies of blood irradiation.

It could be argued that the canine transplantation experiments did not adequately test the hypothesis under question: Even with the best immunosuppressive regimens, it is not possible to obtain chronic survival in more than a small percentage of dogs after renal homotransplantation, a particularly significant disadvantage since thymectomy might be expected to become a factor only late after operation. In rodents thymectomized in adult life, there is a latent period of about 4 months before selective loss of the circulating immunologically competent small lymphocytes becomes obvious, along with a decreased ability to produce certain antibodies (Jeejeebhoy, 1965; Metcalf, 1965; Miller, 1965; Taylor, 1965). The duration of such a transition process is not known in dogs, but in patients one would expect the interval to be more protracted since in man the majority of these lymphocytes in the circulating pool have a life span that is measured in years (Buckton & Pike, 1964).

In the clinical trial of this report in which the criticism of short follow-up did not pertain, there was evidence that the thymus gland could retain immunologic function in adult man or at least that its removal could have a delayed effect upon immunologic responsiveness just as in rodents. When thymectomy was performed prior to renal transplantation, there was a lower incidence and a decreased severity of cellular infiltration and immunoglobulin deposition in the kidney homografts compared to these findings in non-thymectomy cases.

Unfortunately, a benefit from the thymic excision was not translated into a lower mortality. Presumably, other factors were of far greater importance than the presence or absence of the thymus in determining the outcome in the first several years after transplantation. Moreover, within the 3½–5 year span of the present study, the reduction of pathologic damage to the homografts in the thymectomy cases was not reflected by renal function superior to that in the controls. At present, it is sometimes difficult to assess the importance, in terms of eventual renal function, of the histologic abnormalities of the kind that were less advanced and less numerous in the thymectomy series. In particular, the long term significance of the glomerular capillary depositions is not known with certainty. Our present limited experience suggests that the latter kind of lesion is often progressive. If this view is correct, the beneficial effects of thymectomy in the patients still living may become more obvious in future years.

More than a year ago, the preliminary results of the present study were published with emphasis on the failure of thymectomy to improve survival and with the reservation that the pathologic studies had not yet been finished (Starzl *et al.*, 1968). At that time, it was stated that thymectomy as a special step in human organ transplantation did not seem justified. With the more complete information now available, this position must be modified

at least to the extent of suggesting the defensibility of further well controlled clinical trials.

From 1964 to 1966 when the transplantations for this investigation were being performed, there still was an unacceptable death rate during the first 2 years after renal transplantation making it difficult to assess the influence of subtle factors upon the ultimate outcome. Since then, refinements in care including the introduction of heterologous ALG to the therapeutic regimen have changed the complexion of the problem. Now, the overall 1 year survival after this kind of operation has increased to about 90% in our institution. Under these improved circumstances, it may be easier to unmask an effect of thymectomy that has been shown to be uninfluential in the early convalescence, but that may determine the difference between homograft survival for some finite period versus a lifetime. If this can be proved, the benefits of thymectomy would then have to be weighed against the possible adverse consequences which could include a progressive disposition to infection or even to neoplasia.

ACKNOWLEDGMENTS

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